The microenvironment and treatment resistance in chronic lymphocytic leukemia
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Citation for published version (APA):

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Chapter 7

The role of Noxa in two murine models of CLL

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Abstract

An important determinant of drug resistance in chronic lymphocytic leukemia (CLL) is the decreased ability of leukemic cells to undergo apoptosis in the lymph node microenvironment. We have shown previously that expression of the pro-apoptotic protein Noxa is increased in human CLL, but that it is significantly lower in lymph nodes as compared to peripheral blood CLL cells. In order to obtain more insight into the role of Noxa in the pathobiology of CLL, we have ablated Noxa in two murine models for CLL, and assessed the consequences for leukemia development.

First, at the age of 9-12 months Lck-APRIL transgenic (Tg) develop a lymphoid neoplasia originating from the peritoneal B-1 cell population, resembling human CLL. Crossing APRIL Tg mice with Noxa knockout mice (KO) (Noxapril mice) led to an accumulation of B220dim/CD19+ B cells in blood, mesenteric lymph nodes and spleen. In mesenteric lymph nodes, there was a clear trend towards higher levels of B220dim/CD19+ B cells in Noxapril mice as compared to APRIL Tg mice. Second, we crossed Noxa KO and Eμ-TCL1 transgenic mice, the latter representing a well established model for CLL. Ablation of Noxa in this context led to significantly enhanced accumulation of B220dim/CD19+ B cells in the peripheral blood of 8 months old mice. Moreover, survival of Noxa KO/TCL1 mice was clearly shorter than for TCL1 mice. Thus, in the context of overexpression of the established oncogene TCL1, Noxa ablation resulted in enhanced leukemogenesis. These data confirm the notion that Noxa plays a role in the pathobiology of human CLL.
Introduction

Chronic lymphocytic leukemia (CLL) is characterized by an accumulation of monoclonal mature CD5+ B-cells. Two factors are thought to play an important role in the pathogenesis of CLL. First, in CLL cells the balance between pro- and anti-apoptotic proteins is shifted towards an anti-apoptotic profile compared to normal B-cells (1). Second, it has been shown that these monoclonal B-cells proliferate in lymph nodes (LNs) of CLL patients (2). In the LN microenvironment both cell-cell contact as well as soluble factors result in enhanced survival, resistance to cytotoxic drugs and proliferation of CLL cells (3).

In the lymph nodes, CLL cells can interact with various cell types, such as T cells, nurse-like cells (NLCs) and stromal cells (4) which results in enhanced survival of CLL cells. Previously, we have reported that CLL cells derived from LNs show significantly lower expression of the pro-apoptotic protein Noxa as compared to CLL cells derived from peripheral blood, resulting in an anti-apoptotic profile in LNs (5). Noxa is considered to be a weak inducer of apoptosis on its own, but appears to be crucial in inducing cell death by targeting the anti-apoptotic protein Mcl-1 for proteasomal degradation (6, 7). Furthermore, ex vivo LNs also show enhanced NF-κB signaling (8) which is also correlated with increased survival of CLL cells (9, 10). One of the candidates to activate NF-κB and protect CLL cells from apoptosis is a proliferation-inducing ligand (APRIL), which can be secreted by NLCs in vitro (11, 12).

APRIL is a member of the TNF superfamily with homology in structure and function to other cytokines in this family (13). APRIL solely acts as a secreted factor. APRIL has been found to stimulate the growth of tumour cells and the proliferation of primary lymphocytes (13-15). Increased circulating APRIL levels have been found in the serum of CLL patients (16). APRIL binds to two receptors of the TNF superfamily, CD269 (formerly called B cell maturation antigen (BCMA)) and CD267 (formerly called Transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI)) (14, 17, 18). Those receptors, CD269 and CD267, are also expressed on CLL cells and, when bound to APRIL, can induce enhanced survival in vitro (11, 19). Planelles et al showed that aging APRIL transgenic (Tg) mice develop a B-1 cell associated lymphoproliferative disease, which is viewed as a model for human CLL (16). However, tumour formation was not observed in all APRIL Tg mice and clonal expansion of B1 B cells of APRIL Tg mice has not been shown.

Another commonly used murine model for CLL is the Eμ-TCL1 transgenic mouse. These mice are transgenic for the human T cell leukemia 1 (TCL1) gene controlled by the immunoglobulin μ heavy-chain promoter/enhancer and show high expression of TCL1
in mature B cells. Although originally identified in T cell leukemia (20), high levels of TCL1 have been found in a broad variety of human tumour-derived B cell lines and in many cases of B cell neoplasias (21). Eμ-TCL1 transgenic mice, designated as TCL1 Tg mice, develop a clonal CD5+ B lymphoproliferative disease at 8 to 12 months of age (22). TCL1 has been shown to be expressed in human CLL and inhibits activator protein 1 (AP-1) activity, an inducer of apoptosis (23, 24) and activates the pro-survival NF-κB pathway (25).

The aim of the current study was to investigate the role of Noxa in the pathobiology of CLL, by knocking out Noxa in two murine models for CLL, APRIL Tg and TCL1 Tg mice, and assessing the consequences for leukemia development. Furthermore, we compared APRIL Tg and Noxapril as to possible other abnormalities in their B cell compartment and investigated whether APRIL Tg and Noxapril mice showed differential antibody responses upon a T cell independent stimulus.

Materials and Methods

Mice
Noxa knockout (KO) mice were a kind gift from Dr. A. Strasser (WEHI, Melbourne) and provided by Dr. M. Serrano (CNIO, Madrid). APRIL transgenic (Tg) mice were a kind gift from Prof. dr. J. P. Medema (AMC, Amsterdam). Stein et al has been described APRIL transgenic mice previously (26). The APRIL Tg mouse line expresses human APRIL under the lck-distal promoter, which directs transgene expression to mature thymocytes and peripheral T lymphocytes. Noxapril mice were generated by crossing Noxa KO mice with APRIL Tg mice. Eμ-TCL1 transgenic mice (TCL1 Tg) were a kind gift of C.M. Croce (University of California, San Diego). Noxa KO/TCL1 Tg mice generated by crossing Noxa KO mice with TCL1 Tg mice. All mice were housed under conventional barrier protection, in compliance with national guidelines. Experiments were approved by the animal ethical committee of the University of Amsterdam. All mice were either generated in B6 mice or backcrossed at least ten times on this background.

Cell preparation, antibodies and flow cytometry
From spleens, bone marrow (1 femur) and mesenteric lymph nodes single cell suspensions were prepared. Erythrocytes were lysed with ammonium chloride buffer. Single cell suspensions were stained with antibodies (CD19-FITC, CD43b-PE, CD5-PerCP-Cy5.5, CD23-PeCy7, B220-APC, IgM-Alexa, Ki-67-PE, CD138-PE, CD21/35-FITC, IgD-PE) purchased from Beckton Dickinson (San Jose, CA), Beckman Coulter (Woerden, The Netherlands) and eBioscience (San Diego, CA). Anti-CD16/32 (1.25 μg/ml) (clone 2.4G2 Bioceros, Utrecht, The Netherlands) was used to prevent non specific binding of antibodies. Expression of cell surface molecules and intracellular proteins was
determined using the FACSCalibur or FACSCanto flow cytometer (BD biosciences). CellQuest software (Beckton Dickinson) was used for data acquisition. Data were analyzed with FlowJo software (TreeStar, San Carlos, CA, USA). For intracellular Ki-67 expression analysis, cells were fixed and permeabilised (eBioscience, San Diego, CA) and subsequently stained with FITC-conjugated Ki-67 or isotype control (Becton Dickinson, San Jose, CA).

TNP-ficoll immunizations
Mice were immunized with 50 μg trinitrophenyl conjugated to Ficoll (TNP-Ficoll) 15:1 (Biosearch Technologies) in alum. Blood was collected after 7, 14, 21, 28, 35 and 42 days after immunization. Isotype specific ELISA was performed on murine serum.

ELISA
ELISA plates were coated over night with TNP-BSA (Biosource technologies). Antibody levels were determined using biotinylated detection antibodies (Southern Biotech). Biotinylated antibodies were detected using streptavidin labelled alkaline phophatase (Southern Biotech) and SigmaFAST pNPP tablets (Sigma-Aldrich). Isotype specific antibodies were used as standards. Optical density was determined using a photospectrometer.

Statistics and calculation
The Shapiro-Wilk normality test was performed to analyze Gaussian distributions. If there was a Gaussian distribution a two-sided t test was used to analyze differences between the groups. If there was no Gaussian distribution, a two-tailed Mann-Whitney U test was used to analyze differences between the groups and a Wilcoxon matched paired test to analyze differences between paired samples. Statistically significance of the data was set at P < 0.05, with one asterisk (*) representing 0.01 < P < 0.05; two asterisk (**) 0.001 < P < 0.01; three asterisk (***) P<0.001.

Results
APRIL Tg and Noxapril mice show an expansion of B220dim/CD19+ cells in blood, mesenteric lymph nodes and spleen
Previously, it has been shown that at 9 months of age some APRIL Tg mice develop lymph node hyperplasia initiated by B-1 B lymphocyte expansion in the peritoneal cavity (16). B-1 B lymphocytes express CD19, CD5, CD43b and IgM and show low expression of B220 and CD23. These characteristics are comparable to antigen expression patterns of human CLL cells. To investigate the role of the pro-apoptotic protein Noxa in the pathobiology of CLL, APRIL Tg mice were crossed with Noxa KO mice generating so called Noxapril mice. At different time points, cells from peripheral blood, peritoneal cavity, mesenteric lymph nodes and spleens were analysed in APRIL Tg mice as well as
in Noxapril mice. As negative controls, all analyses were also performed on wild type and Noxa KO mice. Previously, it has been shown that Noxa KO mice have no B cell abnormalities under steady state conditions (27). A B220dim/CD19+ population was present in all mice, which showed expression of CD5, CD43b and IgM and was negative for CD23 (Figure 1A). In both 12 months old APRIL Tg and Noxapril mice a significant accumulation of B220dim/CD19+ cells was found in peripheral blood (PB) as compared to wild type mice (Figure 1B). Numbers of B220dim/CD19+ cells from the peritoneal cavity of APRIL Tg mice accumulated over time which has also been shown by Planelles

Figure 1. Analysis of B220dim/CD19+ cells in peripheral blood, peritoneal cavity, mesenteric lymph nodes and spleens of wild type, APRIL Tg, Noxa KO and Noxapril mice. A. A representative FACS plot of B220dim/CD19+ cells from peripheral blood from a Noxapril mouse 12 months of age is depicted. Gating strategy from left to right: % of CD19+ cells; % of B220dim/CD19+ cells; % CD5, CD23, CD43b and IgM expression of B220dim/CD19+ cells. B. Absolute numbers of B220dim/CD19+ cells in peripheral blood of wild type (solid squares), APRIL Tg (open squares), Noxa KO (solid circles) and Noxapril (open circles) mice of 2, 6, 9 and 12 months of age. Averaged results from 3 mice per group are presented in mice 2, 6 and 9 months of age and averaged results from 5 mice per group are presented in mice 12 months of age. C. Absolute numbers of B220dim/CD19+ cells from the peritoneal cavity of wild type, APRIL Tg, Noxa KO and Noxapril mice. Averaged results from 3 mice per group are presented in mice 2 and 6 months of age and averaged results from 8 mice per group are presented in mice 12 months of age. D. Absolute numbers of B220dim/CD19+ cells in mesenteric lymph nodes of wild type, APRIL Tg, Noxa KO and Noxapril mice of 6 (6 mice per group) and 12 months of age (8 mice per group). E. Absolute numbers of B220dim/CD19+ cells from spleens of wild type, APRIL Tg, Noxa KO and Noxapril mice of 3 (3 mice per group), 6 (6 mice per group) and 12 months of age (8 mice per group). Error bars represent the mean ± SEM in Figure 1B-1E. Significance is calculated from wild type versus APRIL TG and Noxapril mice 12 months of age. * .01 < P < .05; ** .001 < P < .01.

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et al (16) (Figure 1C). In contrast, 12 months old Noxapril mice revealed declining levels of peritoneal B220dim/CD19+ cells (Figure 1C). This decline could be explained by the fact that these peritoneal B220dim/CD19+ cells from Noxapril mice cells migrate to the mesenteric lymph nodes, because in these lymph nodes, we observed a trend towards higher levels of B220dim/CD19+ cells in 12 months old Noxapril mice as compared to APRIL Tg mice (Figure 1D). 12 Months old Noxapril and APRIL Tg mice showed statistical significant higher levels of B220/CD19+ cells in the spleen as compared to wild type mice (Figure 1E). Thus, both 12 months old APRIL Tg and Noxapril mice show an accumulation of B220dim/CD19+ cells in blood, spleen and mesenteric lymph nodes. Notably, a trend towards higher B220dim/CD19+ cells in mesenteric lymph nodes of Noxapril mice versus APRIL Tg was observed.

The accumulation of B220dim/CD19+ cells is not caused by enhanced proliferation

In both APRIL Tg and Noxapril mice an accumulation of B220dim/CD19+ cells was observed. Previously, it has been shown that accumulation of B-1 B cells in APRIL Tg mice was not due to enhanced proliferation, but to increased survival as compared to wild type mice (16). Ki67 levels of B220dim/CD19+ cells were measured from peripheral blood and peritoneal cavity of Noxapril, APRIL Tg mice, Noxa KO and wild type mice (at 6 and 12 months of age) (Figure 2). We did not observe significant differences in

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**Figure 2.** The increase of B220dim/CD19+ cells in APRIL Tg and Noxapril mice is not caused by enhanced proliferation. **A.** Intracellular Ki67 staining of B220dim/CD19 cells derived from peripheral blood from wild type (white bars), APRIL Tg (black bars), Noxa KO (light grey bars) and Noxapril mice (dark grey bars) 6 (left) and 12 months of age (right). **B.** Intracellular Ki67 staining of B220dim/CD19+ cells derived from the peritoneal cavity (PEC) from wild type, APRIL Tg, Noxa KO and noxapril mice 6 (left) and 12 months of age (right). For figure 2A,B, no significant differences have been observed between the different groups of mice. Averaged results from 3 mice per group are depicted. Error bars show mean ± SEM.
proliferation rates of B220dim/CD19+ cells from peripheral blood between APRIL Tg, Noxapril mice and wild type mice (Figure 2A). In line with earlier studies, we did not find significant differences in Ki67 levels of peritoneal B220dim/CD19+ cells from 6 months old APRIL Tg mice versus wild type mice (Figure 2B). Lower levels of Ki67 were observed in peritoneal B220dim/CD19+ cells from Noxa KO and Noxapril mice compared to wild type and APRIL Tg mice but these differences were statistically not significant (Figure 2B). In 12 months old mice, a trend towards higher Ki67 levels was observed in peritoneal B220dim/CD19+ cells in APRIL TG mice versus wild type mice (Figure 2B). Taken together, these data suggest that B220dim/CD19+ cells accumulate in APRIL Tg and Noxapril mice due to enhanced survival rather than due to increased proliferation.

Enhanced levels of plasma cells in APRIL Tg and Noxapril mice

It has been established that APRIL is important for the survival of plasmablasts (28). Thus, we hypothesized there might be an increase of plasma cells in ageing APRIL Tg and Noxapril mice. Indeed, we observed higher levels of plasma cells (B220dim/CD138+) in spleens and mesenteric lymph nodes in both APRIL Tg and Noxapril mice as compared to wild type mice (Figure 3A, B). A trend towards higher numbers of plasma cells was observed in Noxapril mice as compared to APRIL Tg mice (Figure 3A,B). Furthermore, immature B cell subsets were investigated. In mice 6 months of age no differences were observed in Transitional1 or 2 (T1 or T2), marginal zone and follicular B cells between wild type, Noxa KO, APRIL Tg and Noxapril mice (Figure 3C, D). In summary, when focussing on different subsets of B cells in aging mice, we observed higher levels of plasma cells in spleens and mesenteric lymph nodes of APRIL Tg and Noxapril mice as compared to wild type mice. Levels of immature B cells subsets were equal in all groups of mice.

Both APRIL Tg and Noxapril mice show an increased T cell independent type 2 response

Previously, it has been shown that antigen specific IgM levels significantly increase after a T- cell independent immune response in APRIL Tg mice (26). Furthermore, Hardenberg et al showed that APRIL plays an important role in class switching to IgA (29). In addition, the B-1 B cell population, which is expanded in APRIL Tg mice, is involved in antibody response to T cell independent antigens. In Noxa KO mice IgM responses were similar to wild type mice immunized with T cell independent antigens (27). Mice 6-8 weeks of age were immunized with a single intraperitoneal injection of trinitrophenyl conjugated to Ficoll (TNP-Ficoll). In agreement with previous reports, IgM-αTNP levels increased at day 7. APRIL Tg mice showed increased IgM-αTNP levels as compared to wild type mice (Figure 4) (26, 29). However, similar levels of IgM-αTNP were observed in Noxapril mice as compared to APRIL Tg mice (Figure 4).
Noxa KO/TCL1 mice have significantly higher B220/CD19+ B cell counts and shorter survival than TCL1 TG mice.

In order to further investigate the role of Noxa in CLL biology, we investigated whether ablation of Noxa influenced the accumulation of B220dim/CD19+ cells in TCL1 Tg mice which is another widely used in vivo model for CLL. Noxa KO mice were crossed with

Figure 3. APRIL Tg and Noxapril mice 12 months of age show enhanced levels of plasma cells in the spleen and mesenteric lymph nodes. A. Absolute numbers of plasma cells (B220-/CD138+) in the spleen of wild type (solid squares), APRIL Tg (open squares), Noxa KO (solid circles) and Noxapril (open circles) mice of 2 (n=3), 6 (n=6) and 12 (n=8) months of age are shown. B. Absolute numbers of plasma cells (B220-/CD138+) in mesenteric lymph nodes of wild type, APRIL Tg, Noxa KO and Noxapril mice of 6 (n=6) and 12 (n=8) months of age are shown. For Figure A-B error bars represent the mean ± SEM. C. Absolute numbers of transitional 1 (T1) B cells (B220+, IgD-, CD21/35-, IgM+) (left) and transitional 2 (T2) B cells (IgD+, B220+, IgM+) (right) in the spleen of wild type (white bars), APRIL Tg (black bars), Noxa KO (light grey bars) and Noxapril mice (dark grey bars). D. Absolute numbers of marginal zone B cells (B220+, IgD-,CD21/35+, IgM+) (left) and follicular B cells (B220+, IgD-, CD21/35-, IgM-) (right) in the spleen of wild type, APRIL Tg, Noxa KO and Noxapril mice. For Figure 3C-D each bar represents 6 mice per group and mean ± SEM.
TCL1 Tg mice (here noted as Noxa KO/TCL1 Tg mice). As a negative control, peripheral blood of wild type and Noxa KO mice were analysed. Peripheral blood of 4 months old mice showed increased levels of B220dim/CD19+ cells in both TCL1 Tg and Noxa KO/TCL1 Tg mice compared to wild type mice (Figure 5A). Interestingly, 8 months old Noxa KO/TCL1 Tg mice showed significantly higher percentages of B220dim/CD19+ cells in peripheral blood compared to TCL1 Tg mice (Figure 5A). Furthermore, survival of Noxa KO/TCL1 Tg mice was significantly shorter than for TCL1 Tg mice (Figure 5B), which

Figure 4. Enhanced levels of IgM after a T cell independent stimulus in APRIL Tg and Noxapril mice. Serum IgM levels at day 7, 14, 21, 28, 35 and 42 were determined by ELISA from 5 wild type (white bars), APRIL Tg (black bars), Noxa KO (light grey bars) and Noxapril mice (dark grey bars) after immunization with 25 μg TNP-ficoll at day 0. Error bars represent the mean ± SEM.

Figure 5. Analysis of B220dim/CD19+ cells in peripheral blood of wild type, TCL1 Tg, Noxa KO and Noxa KO/TCL1 Tg mice. A. Percentages and absolute numbers of B220dim/CD19+ cells in peripheral blood of wild type (solid squares), TCL1 Tg (open squares), Noxa KO (solid circles) and NoxaKO/TCL1 TG (open circles) mice of 4 and 8 months of age. Averaged results from 7 mice per group are presented. Error bars show mean ± SEM. * .01 < P < .05; ** .001 < P < .01; *** P < .001. B. Survival (Kaplan-Meier) plots of cohorts of 7 mice of the indicated strains. Mean ± SD survival was for TCL1 Tg mice 405.4±42.8 and for NoxaKO/TCL1-Tg 344.7±29.1. Differences in survival between TCL1 Tg and NoxaKO/TCL1 Tg were statistically significant, p= 0.009. More than 50% of WT and NoxaKO mice were still alive at the end of the 500 days observation period.
was accompanied by enlarged spleens (data not shown). These data indicate that in the presence of Noxa, the leukemogenic properties of transgenic TCL1 are at least partially suppressed.

**Discussion**

Transgenic mouse models are an invaluable tool for analysis of the role of specific genes in the pathogenesis of a disease. In this study, we investigated the role of Noxa in the pathobiology of CLL via Noxa ablation in APRIL and TCL1 Tg mice. Although, we observed an accumulation of B220dim/CD19+ B cells in various organs of APRIL Tg mice crossed with Noxa KO mice (Noxapril mice), in the limited number of mice studied these levels were not significantly different when compared to APRIL Tg mice. In addition, we observed higher levels of plasma cells in both aged APRIL Tg and Noxapril mice confirming a role for APRIL in plasma cell survival in vivo. Although these analyses are still at a preliminary stage, in NoxaKO/TCL1 Tg mice, we observed an increase in B220dim/CD19+ cells in peripheral blood and increased mortality, indicating that Noxa partially suppresses leukemic outgrowth in TCL1 Tg mice.

Based upon the phenotypic characteristics of human CLL cells (CD5+ B cells) a parallel has been drawn with specific CD5+ B1 cells in mice. Several mouse models have been described which develop a clonal CD5+ B lymphoproliferative disease, such as TCL1, APRIL, BAFF/TCL1 and TRAF2DN/Bcl-2 transgenic mice (16, 22, 30, 31). In this study, by backcrossing Noxa KO mice with either APRIL or TCL1 transgenic mice, we showed that Noxa ablation enhanced leukemic outgrowth in TCL1 Tg mice, but not in APRIL Tg mice. This might be explained by the fact that the absence of Noxa and overexpression of APRIL have similar effects, i.e. both leading to enhanced survival, whereas TCL1, being an oncogene, can function as a driver for leukemia development (32). In order to substantiate this explanation, more studies into the proportion of proliferating versus apoptotic cells in the various mouse strains should be performed. Ectopic expression of TCL1 driven by the lck promoter in the T cell compartment as well as overexpression of TCL1 under control of the Eμ promoter which specifically targets the B cell compartment, leads to development of leukemia (22, 32). TCL1 Tg mice develop a CD5+ lymphoproliferative disease at younger age when compared to APRIL Tg mice. In addition, the CD5+ B cell expansion in TCL1 Tg mice is monoclonal (22) and this has not been shown for CD5+ B cells in APRIL Tg mice. Currently, we are studying the aspect of clonality in the different strains.

The pro-apoptotic protein Noxa has clearly not been identified as a tumour suppressor gene since Noxa KO mice do not display enhanced levels of B or T cells under steady state conditions. We have previously shown that Noxa ablation provides a survival advantage
for activated B and T cells of low affinity in vitro and in vivo (27, 33). Furthermore, enhanced expression of Noxa was detected in peripheral blood samples of human CLL (1). Based upon these findings, we hypothesized that Noxa might affect leukemogenesis by suppressing the expansion of clonal CD5+ B cells in TCL1 Tg mice. Indeed, we observed a significant increase in CD5+ B cells in NoxaKO/TCL1 Tg mice compared to TCL1 Tg mice, combined with increased mortality. In normal lymphocytes, antigen stimulation leads to B cell expansion and upregulation of Noxa (27). Extrapolating from this, we hypothesize that the increased expression of Noxa in human CLL (1) reflects an elimination stage in normal B cell differentiation which is deregulated upon leukemic transformation. In CLL lymph nodes, Noxa levels appear to be actively suppressed (5) and this could be an important aspect in the leukemic outgrowth. Interaction of CLL cells with other cells residing in the lymph node microenvironment, such as T cells and NLCs, might lead to activation of signaling pathways which suppress Noxa in CLL cells.

Recently, Enzler et al showed that BAFF/ TCL1 Tg mice develop a CLL-like disease at a significantly younger age and have a more aggressive disease and shorter survival than TCL1 Tg mice (30). Enhanced activation of NF-κB has been observed in CD3-/CD5+ cells from TCL1 Tg stimulated with CD257. The NF-κB pathway has been shown to play an important role in enhanced survival of CLL cells in the lymph node microenvironment (8, 10). APRIL is also known to activate the NF-κB pathway in vitro (11). Whether CD5+ B cells from APRIL Tg mice show increased NF-κB activity compared to wild type mice has not yet been explored. In addition, the question arises whether crossing APRIL Tg mice with TCL1 Tg mice results in enhanced NF-κB activation of CD5+ B cells and thereby results in a CLL-like model similar to the BAFF/TCL1 Tg mice. We are currently investigating the phenotype of APRIL/TCL1 Tg mice and observe a CLL-like phenotype which is comparable to BAFF/TCL1 Tg mice (J. Schot, unpublished data).

In conclusion, we here show that in TCL1 Tg mice, which have a more aggressive CLL-like phenotype as compared to APRIL Tg mice, Noxa ablation has enhancing effects on the accumulation of CD5+ B cells and mortality. Although further studies are required to substantiate these findings, this study indicates a role for Noxa in the pathobiology of CLL.
Reference List


